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THE DEPENDENCE OF FORCE AND SHORTENING VELOCITY ON SUBSTRATE CONCENTRATION IN SKINNED MUSCLE FIBRES FROM *RANA TEMPORARIA*

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SUMMARY

1. The force–velocity relation was determined in fully activated skinned fibres from frog muscle at concentrations of the substrate, magnesium adenosine triphosphate (MgATP), ranging from 10 μM to 10 mM. The ionic strength of the solutions was 200 mM, temperature 0–5 °C, pH 7·1. The activation procedure of Moisescu (1976) was used to raise the calcium concentration rapidly in the interior of the fibres. A re-phosphorylating system (creatine kinase and creatine phosphate) was used to maintain the MgATP concentration in the fibres.

2. Isotonic releases were performed using a fast servo-controlled motor and tension transducer. Releases to a pre-determined tension level relative to the isometric tension were made using a novel normalizing circuit.

3. In some of the experiments changes of sarcomere length were recorded using the diffraction device described in the preceding paper (Goldman & Simmons, 1984). There was satisfactory agreement between velocities determined from the total length change and the sarcomere length change.

4. The isometric tension showed a biphasic dependence on MgATP concentration. Tension increased with MgATP concentration from 1 μM to reach a peak at about 30–100 μM and decreased by about 20% from the value at the peak with further increase in the MgATP concentration to 5 mM (about the physiological concentration). At 5 mM-MgATP, the isometric tension was approximately the same as in intact fibres, if allowance is made for the increase in cross-sectional area that occurs when the surface membrane is removed.

5. The maximum velocity of shortening, V_{max}, was obtained by fitting the force–velocity relation using Hill's (1938) equation. V_{max} showed a roughly hyperbolic dependence on MgATP concentration, with a K_m of 0·47 mM. At 5 mM-MgATP, the value of V_{max} was 2·16 muscle lengths per second, which is similar to that of intact fibres.

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6. a/P_0, the parameter of Hill's (1938) equation that is related to the curvature of the force–velocity relation, showed a slight decrease with increasing MgATP concentration. Its value at 5 mM-MgATP of 0·16 is somewhat lower than found for intact fibres.

7. The results are discussed in terms of a simple model based on the biochemical cycle of hydrolysis of ATP by actomyosin in solution. The decrease of tension from about 30 μM to higher concentrations of MgATP can be related to the dissociating effect of MgATP on actomyosin. The increase of isometric tension from 1 to 30 μM-MgATP is discussed in terms of two types of rigor attachment of cross-bridges which support different amounts of tension.

8. At low MgATP concentration shortening velocity appears to be limited by the rate of detachment of cross-bridges from the rigor state. An estimated value for the second order rate constant for detachment by MgATP is in good agreement with the corresponding value for the dissociation of myosin from actin. The saturation shown by V_{max} for MgATP concentrations in the millimolar range is however not apparent in the solution studies. This may indicate that in the physiological cross-bridge cycle the product dissociation step is rate limiting.

INTRODUCTION

Studies of the steady-state and transient mechanical properties of tetanically stimulated intact fibres from frog muscle have strongly suggested that there are a number of distinct transitions involved in the cyclic interaction of cross-bridges with actin sites (see Huxley, 1974). A direct demonstration of the existence of elementary transitions, between biochemical intermediates in the hydrolysis of magnesium adenosine triphosphate (MgATP), has come from kinetic measurements of the interaction between myosin and actin in solution using proteins from rabbit muscle (recently reviewed by Taylor, 1979 and Sleep & Smith, 1981) and from frog muscle (Ferenczi, Homsher, Trentham & Simmons, 1978).

In order to establish the relation between the different states of the cross-bridge implied by the mechanical measurements and those observed in the biochemical experiments, we have undertaken a study of the mechanical properties of frog muscle fibres at different concentrations of the substrate MgATP. One aim of these experiments is to determine the dependence on MgATP concentration of the various 'mechanical' rate constants and to correlate this dependence where possible with corresponding effects known from the biochemical measurements.

In this paper we describe the results of experiments on the steady-state isometric tension and shortening velocity. The maximum shortening velocity is of particular interest as it is likely to be the mechanical state which most closely resembles the unhindered interaction of myosin with actin in solution. In subsequent papers we shall deal with the effects on shortening velocity of competitive inhibitors of the actomyosin ATPase and with the dependence on MgATP concentration of the tension transients produced by rapid changes of length.

It is not possible to vary the intracellular concentration of MgATP in intact fibres in a controlled manner, so we have used the 'skinned' fibre preparation (Natori, 1954; Hellam & Podolsky, 1969) in which the surface membrane of a length of fibre is removed by dissection. The fibre segment can then be activated in the presence of

a desired concentration of MgATP by increasing the calcium ion concentration in the bathing solution.

Some of these results have been communicated to the Physiological Society (Ferenczi, Goldman & Simmons, 1979).

METHODS

The methods for preparing fibres and the apparatus were as described by Goldman & Simmons (1984).

Solutions. The constituents of the solutions were calculated by a computer program which used affinity constants from the literature to solve the multiple binding equilibria of the components. Table 1 shows the composition of the solutions except for a few intermediate MgATP concentrations which can be obtained by interpolation.

All the solutions were at pH 7·1 at 0 °C and the calculated ionic strength was maintained at 200 mM by altering the concentration of either creatine phosphate or HDTA. During an experiment, the solution in which the fibre was immersed was stirred continuously to achieve an even temperature (0–5 °C) in the trough and to minimize diffusional boundary layers. Stirring was stopped for a few seconds just before applying a tension step. The EGTA buffer gradient method of Moisescu (1976) was used to change the calcium ion concentration rapidly in order to minimize the activation time in each contraction as described by Goldman & Simmons (1984).

Isotonic releases. During activation by calcium the fibre was held isometric while force developed. When a steady isometric tension was reached (after about 2–3 s at full activation), the tension on the fibre was lowered rapidly (within 10 ms) to the required isotonic value and the velocity of the steady shortening was measured a few milliseconds after the tension had settled to a steady value. The isometric tension varied considerably in the different activating solutions used. In comparing shortening velocities in different solutions, the isotonic releases were made to the same tension relative to the isometric value for the same contraction using an electronic circuit. This tension-normalizing circuit is shown in a simplified form in Fig. 1.

Operational amplifiers A1 and A2 and a multiplying digital-to-analog converter formed a variable-gain non-inverting amplifier. The multiplying digital-to-analog converter was an integrated circuit which generated a current (I_{out}) proportional to an analog voltage (V_{ref}) multiplied by the binary value of a digital scaling factor. Thus, the multiplying digital-to-analog converter acted as a resistor, whose value was set by the logic levels of the bits B1 to B12. This resistor was placed in the feed-back loop of the operational amplifier A2 so that the amplification was determined by the digital output of a binary up/down counter.

When a steady level of tension was reached during a contraction of the muscle fibre, a series of clock pulses were applied to the up/down counter. The direction of counting of this digital register was determined by a comparator circuit which was referenced to a voltage level of +1 V. The output of the comparator was connected to the direction input (up/down) of the counter, so that the clock pulses caused the counter to increment or decrement until the amplification of A2 caused the normalized tension signal (T_{norm}) to reach +1 V. The clock pulses stopped and the output of A2 was then a tension signal normalized to +1 V. The zero tension value of T_{norm} was set before the contraction to 0 V by a steady zero offset voltage generated by a similar counter, digital-to-analog converter and comparator combination (not shown).

For isotonic feed-back, a tension error signal T_{error} was generated by A3 which summed the T_{norm} signal, a steady −1 V level and a tension step pulse. Potentiometer P1 set the feed-back gain in the isotonic feed-back mode of the apparatus. After completion of the tension normalization, the feed-back for the motor was smoothly switched by a control ramp from the normal motor error signal to T_{error} by two analog multipliers AM1 and AM2 and the amplifier A4. When the control ramp input was at +5 V, the output of AM2 was zero and AM1 and A4 passed the motor error signal to the motor power amplifier for isometric control of the fibre length. As the control ramp voltage decreased towards −5 V, the motor error signal was attenuated by AM1 and T_{error} was allowed through to the motor amplifier by AM2 and A4. When the control ramp reached −5 V, the output of AM1 was zero and the motor was controlled fully by the tension signal. Tension normalization required about 20 ms and the subsequent change-over to tension control was accomplished in about 200 ms.

When the muscle was thus in tension-control mode, the tension step input and the T_{error} signal

TABLE 1. Composition of solutions: intermediate solutions can be obtained by interpolation

Solution name	Final MgATP	Free Mg^{2+}	Total Na_2ATP	Total $MgCl_2$	Total EGTA	Total Ca	Total HDTA	Total CP	Total TES
				Standard solutions					
Relaxing	0·010	1·0	0·0118	2·1	30	—	—	29·13	100·0
Pre-activating	0·010	1·0	0·0118	1·6	0·1	—	29·90	29·41	100·0
Activating	0·010	1·0	0·0119	1·35	30	29·81	—	29·1	100·0
Relaxing	0·100	1·0	0·118	2·18	30	—	—	28·95	100·0
Pre-activating	0·100	1·0	0·118	1·69	0·1	—	29·90	29·23	100·0
Activating	0·100	1·0	0·119	1·45	30	29·81	—	29·28	100·0
Relaxing	0·300	1·0	0·353	2·38	30	—	—	28·54	100·0
Pre-activating	0·300	1·0	0·353	1·88	0·1	—	29·90	28·82	100·0
Activating	0·300	1·0	0·357	1·65	30	29·81	—	28·87	100·0
Relaxing	0·500	1·0	0·587	2·57	30	—	—	28·12	100·0
Pre-activating	0·500	1·0	0·588	2·08	0·1	—	29·90	28·41	100·0
Activating	0·500	1·0	0·595	1·84	30	29·81	—	28·46	100·0
Relaxing	1·0	1·0	1·17	3·06	30	—	—	27·1	100·0
Pre-activating	1·0	1·0	1·17	2·56	0·1	—	29·90	27·38	100·0
Activating	1·0	1·0	1·19	2·33	30	29·81	—	27·42	100·0
Relaxing	5·0	1·0	5·86	6·97	30	—	—	18·91	100·0
Pre-activating	5·0	1·0	5·86	6·47	0·1	—	29·90	19·19	100·0
Activating	5·0	1·0	5·94	6·23	30	29·81	—	19·18	100·0
Relaxing	10·0	1·0	11·68	11·8	30	—	—	8·74	100·0
Pre-activating	10·0	1·0	11·69	11·3	0·1	—	29·90	9·02	100·0
Activating	10·0	1·0	11·84	11·1	30	29·81	—	8·92	100·0
				Solutions containing extra creatine phosphate					
Relaxing	0·5	1·14	0·588	2·57	10·0	—	—	48·14	100·0
Pre-activating	0·5	0·964	0·588	2·08	0·1	—	9·90	48·42	100·0
Activating	0·5	0·871	0·595	1·84	10·0	10·0	—	48·47	100·0
				Solutions with excess free ATP					
Relaxing	0·3	0·009	5·65	0·318	30	—	—	16·4	100·0
Pre-activating	0·3	0·009	5·65	0·313	0·1	—	29·90	16·55	100·0
Activating	0·3	0·009	6·15	0·311	30·3	30·03	—	16·15	100·0
Relaxing	0·5	0·015	5·98	0·53	30	—	—	15·94	100·0
Pre-activating	0·5	0·015	6·03	0·52	0·1	—	29·90	16·77	100·0
Activating	0·5	0·016	5·96	0·52	30·3	30·0	—	16·18	100·0
Relaxing	5·02	0·165	10·00	5·32	30	—	—	9·646	100·0
Pre-activating	5·01	0·165	10·00	5·24	0·1	—	29·90	9·837	100·0
Activating	4·78	0·165	10·00	4·96	30·3	30·0	—	10·37	100·0
Solution name	Final MgATP	Free Mg^{2+}	Total Na_2ATP	Total $MgCl_2$	Total EGTA	Total Ca	Total HDTA	Total NaADP	Total TES
				Solutions without creatine phosphate					
Relaxing	0·5	1·0	0·57	2·47	30·0	—	28·71	—	100·0
Pre-activating	0·5	1·0	0·57	1·97	0·1	—	58·89	—	100·0
Activating	0·5	1·0	0·58	1·73	30·0	30·0	29·04	—	100·0
Relaxing	5·0	0·998	5·859	6·89	30·0	—	19·5	—	100·0
Pre-activating	5·0	0·996	5·86	6·47	0·1	—	49·68	—	100·0
Activating	5·0	1·01	5·938	6·16	30·0	29·85	19·76	—	100·0

TABLE 1 (*Cont.*)

Solution name	Final MgATP	Free Mg^{2+}	Total Na_2ATP	Total $MgCl_2$	Total EGTA	Total Ca	Total HDTA	Total NaADP	Total TES
				Same, but with 2 mM-MgADP					
Relaxing	0·5	0·980	0·58	4·40	30·0	—	20·13	—	100·0
Pre-activating	0·5	0·975	0·58	3·90	0·1	—	50·32	—	100·0
Activating	0·5	0·973	0·58	3·67	30·0	30·0	20·45	—	100·0
Relaxing	4·93	1·03	5·80	8·83	30·0	—	10·85	6·34	100·0
Pre-activating	4·93	1·04	5·80	8·33	0·1	—	41·04	6·34	100·0
Activating	4·93	1·04	5·87	8·09	30·0	29·82	11·10	6·39	100·0

All values are in mM.

TES: *N*-tris[hydroxymethyl]methyl-2-aminoethanesulphonic acid.

HDTA: 1,6-diaminohexane-*N*,*N*,*N*′,*N*′-tetraacetic acid.

EGTA: ethyleneglycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid.

CP: creatine phosphate.

All solutions also contained approximately 44 mM-free sodium ions and 90 mM-free potassium ions. The potassium ions accompanied the TES, HDTA and EDTA stocks, chloride came with the added magnesium ions and CP, and the sodium ions accompanied the CP and nucleotide stocks. All activating solutions had a pCa in the range 4·5–4·7, except where noted in the text. All solutions containing creatine phosphate had, in addition to the constituents described above, 1 mg ml^{-1} of creatine kinase (CPK) except where noted in the text. Fast relaxing solution consisted of relaxing solution to which extra 20 mM-EGTA was added (Goldman & Simmons, 1984).

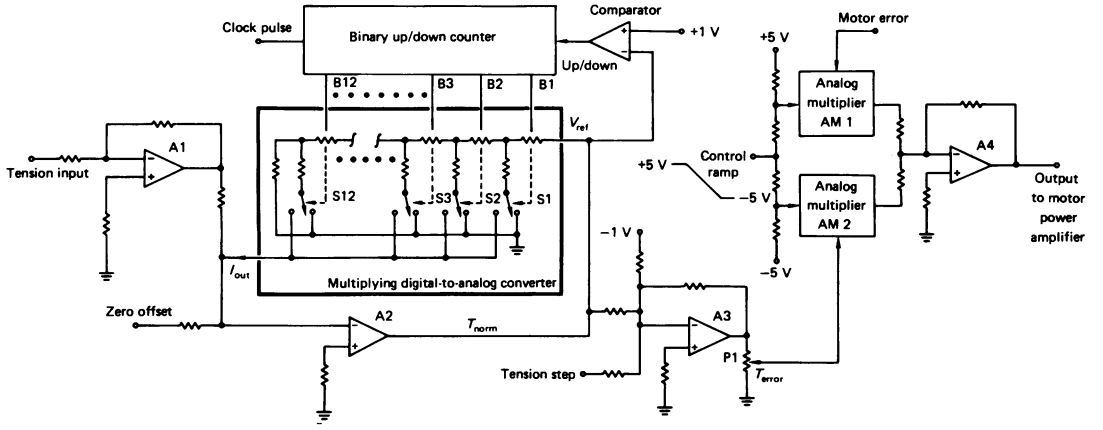


Fig. 1. Simplified diagram of tension normalization and isotonic control system. For mode of operation, see text. Operational amplifiers A1–A4 were Precision Monolithics OP–02. The multiplying digital-to-analog converter was Analog Devices AD7531. The binary up/down counter (12 bits) was made by cascading three Texas Instruments 74193 4-bit integrated counter circuits. The comparator was a Precision Monolithics CMP-01. Analog multipliers AM1 and AM2 were Analog Devices AD534. Another amplifier, multiplying digital-to-analog converter, up/down counter and comparator (referenced to 0 V) combination generated the zero offset signal to set the T_{norm} signal automatically to 0 V before each contraction.

were zero so the muscle was still held at the isometric tension level. About 1 s was allowed so that the status of the muscle in the isometric tension-control mode could be monitored. Then a predetermined voltage was applied to the tension step input to reduce the tension to an isotonic test value for measurement of shortening velocity. Since the isometric tension corresponded to +1 V at A2 regardless of the actual tension, a standard voltage step applied to the tension step input

caused the tension to approach a fixed fraction of the isometric tension. This facilitated comparison of shortening velocity at each relative tension as the MgATP concentration was varied.

The fibre shortened until the servo system reached a pre-determined electronic stop, when control was returned to the motor position signal. This was done using a diode switching network similar to that of Gordon, Huxley & Julian (1966). Isometric tension then redeveloped, the fibre was transferred to relaxing solution, allowed to relax fully and then was restored to its original length. In some of the experiments, the sarcomere length was recorded in addition to the motor position and tension, using the diffraction system described previously (Goldman & Simmons, 1984).

The primary interest in these experiments was in the steady-state tension and shortening velocity and for this reason we did not attempt to perform very rapid isotonic releases. Most of the records in this paper show a small overshoot in the tension records after an isotonic release before a steady level was reached. This was due to a slightly incorrect setting of the servo system parameters.

Experimental procedure. The fibre was mounted on the mechanical apparatus and measurements were made of its length (l), mean sarcomere length (s) and of its cross-sectional area (A) as described by Goldman & Simmons (1984).

The first contraction of a fibre was performed in the 5 mM-MgATP reference solution. The fibre was then placed in a relaxing solution containing a different (test) concentration of MgATP and five minutes or more were allowed for equilibration. The next two contractions were done in activating solution at the test MgATP concentration. The fibre was then returned to the 5 mM-MgATP solutions for the next two contractions, and so on. Fibres gave ten to eighteen useful contractions before tension fell to below 80% of the original value or became visibly irregular when viewed with a dissecting microscope. Thus, it was usually possible to compare the performance of a fibre in the reference and one test MgATP solution, at about four to five different isotonic tension levels. The isotonic tension level was altered every two cycles of contraction so that the same level was used in the reference and the test solution in successive contractions. The experiments were usually done using a starting sarcomere length of 2·6 μm so that the period of steady shortening occurred at a sarcomere length greater than 2·0 μm.

The isotonic levels used were typically 0·5 P_0, 0·25 P_0, 0·13 P_0 and 0·01 P_0, where P_0 is the isometric tension level. A control experiment showed that the values of V_{max} and a/P_0 (see below) based on shortening at these levels agreed well with the values obtained from a larger number of data points.

Analysis of data. Signals proportional to tension, motor position (p_M, corresponding to changes in over-all fibre length) and, in some experiments, sarcomere length (s) were recorded on a storage oscilloscope and photographed with 35 mm or Polaroid film. In the later stages of this work a computer (PDP 11–34, Digital Equipment Corporation) was used to store the data on magnetic disk.

Shortening velocity (V) was expressed in terms of muscle lengths per second, referred to a sarcomere length of 2·25 μm. To compensate for variability between contractions, the isotonic tension was expressed relative to the isometric tension in the same contraction. The force–velocity data for each fibre at a given MgATP concentration were fitted by a hyperbola (Hill, 1938):

$$(P+a)(V+b) = b(P_0+a),$$

where P is the isotonic tension during shortening, V is the shortening velocity, P_0 is the isometric tension, and a/P_0 is a measure of the curvature of the hyperbola and a and b are constants. The maximum velocity of shortening (V_{max}) at zero load ($P = 0$) is equal to $b P_0/a$.

The values of a/P_0 and V_{max} which minimized the sum of the squared differences of velocity between the experimental data from a fibre and the above equation were found by a method based on Wilkinson (1961).

RESULTS

Force–velocity curve at 5 mM-MgATP. Fig. 2*A* shows tension records for two contraction–relaxation cycles which were used to determine shortening velocity in 5 mM-MgATP. After transfer to activating solution (arrows b and k), tension developed to reach a steady level (P_0); the time taken for the tension to reach half its isometric value was about 1·2 s. The arrows c and l indicate the times when the fibre entered tension control. At points d and m tension was rapidly reduced to 0·15 P_0 and 0·04 P_0 respectively.

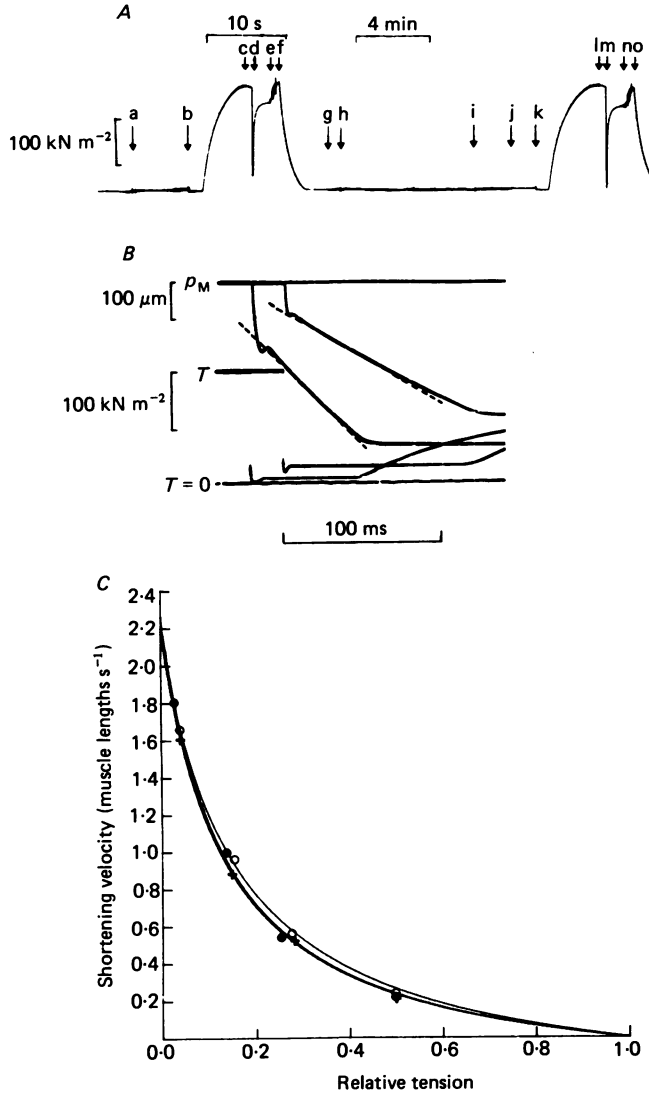


Fig. 2. Force–velocity relation in 5 mM-MgATP. *A*, pen recorder traces of tension in eighth and ninth contractions after skinning. Fibre initially in relaxing solution under length control, recorder speed 5 mm min^{-1}. At a and also i, the fibre was transferred to pre-activating solution. At b and j the recorder speed was increased to 125 mm min^{-1}. The transfer to activating solution was started at b and k. At c and l, the motor control was switched to the tension signal. At d and m, the fibre was released isotonically to 0·15 P_0 and 0·04 P_0 respectively. The fibre shortened 350 and 450 μm respectively, then was held isometric and tension redeveloped. At e–f and n–o the fibre was transferred to fast relaxing solution, then to relaxing solution (h and q). The recorder speed was changed to 5 mm min^{-1} at g and p. *B*, oscilloscope records of tension (T) and length (p_M) for the same contractions. *C*, force–velocity relation for same fibre at three different starting sarcomere lengths. Open circles, 2·33 μm; crosses, 2·45 μm; filled circles, 2·74 μm. Fibre dimensions: l = 3·62 mm, s = 2·71 μm, $A = 1{\cdot}14 \times 10^{-8}$ m^2. Temperature = 2·7 °C.

Fig. 2*B* shows the same contractions on a faster time base, together with the length records. When the tension was suddenly reduced from the isometric to the test level a simultaneous decrease in length was observed. When the tension had stabilized at the test value the fibre shortened at a fairly steady rate. The dashed lines show the tangents to the length traces that were used to measure shortening velocity. The fibre shortened a pre-set distance before the electronic shortening stop was reached, at which point the fibre was automatically switched back to the length-control mode and tension redeveloped. The fibre was then placed in relaxing solution (points f and o).

The contractions shown in Fig. 2 were part of an experiment to study the effect of sarcomere length on the shortening velocity. The shortening velocities for a number of isotonic releases were measured in contractions at different starting sarcomere lengths. The resulting force–velocity relations are shown in Fig. 2*C*. There was little difference between the values of V_{max} and a/P_0 for the three starting sarcomere lengths; for 2·33, 2·45 and 2·74 μm, the values of V_{max} were 2·17, 2·18 and 2·26 muscle lengths per second respectively, and the values for a/P_0 were 0·16, 0·13 and 0·13 respectively.

In many of the experiments the velocity decreased as the shortening progressed, as reported previously for skinned fibres by Brenner (1980). The decrease of velocity became more pronounced in the course of an experiment, but provided the measurement of velocity was made soon after the isotonic release there was no appreciable difference in the values obtained in the same solution at the beginning and end of an experiment. In a more recent analysis of similar data in this laboratory the shortening traces were fitted with a polynomial and the velocity was obtained from the slope of the polynomial extrapolated to the time of the isotonic release. There was little difference in the values obtained by this method and the one used in the present study.

Shortening velocity was not expected to be markedly influenced by extra compliance at the ends of the fibre since the stress on the end compliance was constant during the isotonic phase of the contraction. This expectation was confirmed by direct measurements of the sarcomere shortening with the laser diffraction device described in the preceding paper (Goldman & Simmons, 1984). Fig. 3 shows muscle length, sarcomere length and tension records for this type of experiment which was done at partial activation to preserve the uniformity of the sarcomeres. The muscle fibre was activated at a pCa of about 5·7, a calcium concentration which gave 65% of the maximal isometric tension for this fibre. Evidence of the presence of end compliance is apparent on the sarcomere length record(*s*) in Fig. 3*B* which continues to decrease after the over-all length trace (p_M) becomes horizontal and tension redevelops. Nevertheless, the force–velocity curves obtained from the motor deflexion (Fig. 3*C*) and from the sarcomere length signal (Fig. 3*D*) are very similar.

In experiments using the diffraction device, the amount of slowing of the motor position trace and the sarcomere length trace during the isotonic phase was about the same, indicating that the cause of the slowing did not lie in abnormal behaviour in the damaged ends of a fibre. When the velocity was measured early in the isotonic phase of the contraction, the force–velocity relation showed little effect of the degree of activation on V_{max} or a/P_0. However, as shown by Brenner (1980), the slowing of the shortening speed was more pronounced at partial activation than at full

activation, so that measurements of velocity late in the isotonic phase would have led to the conclusion that shortening velocity decreases with decreased activation.

Isometric tension was correlated with fibre cross-sectional area. In 5 mM-MgATP and at full calcium ion activation, the isometric tension level was $148+9$ kN m^{-2} (mean $\pm$ S.E. of mean, $n = 41$ fibres) at an average sarcomere length of $2{\cdot}56 \pm 0{\cdot}02$ μm. The average V_{max} in the same conditions was $2{\cdot}16 \pm 0{\cdot}10$ muscle lengths per second (mean $\pm$ S.E. of mean, $n = 38$) referred to a sarcomere length of 2·25 μm. The mean value of a/P_0 was $0{\cdot}16 \pm 0{\cdot}01$ for the same series of fibres.

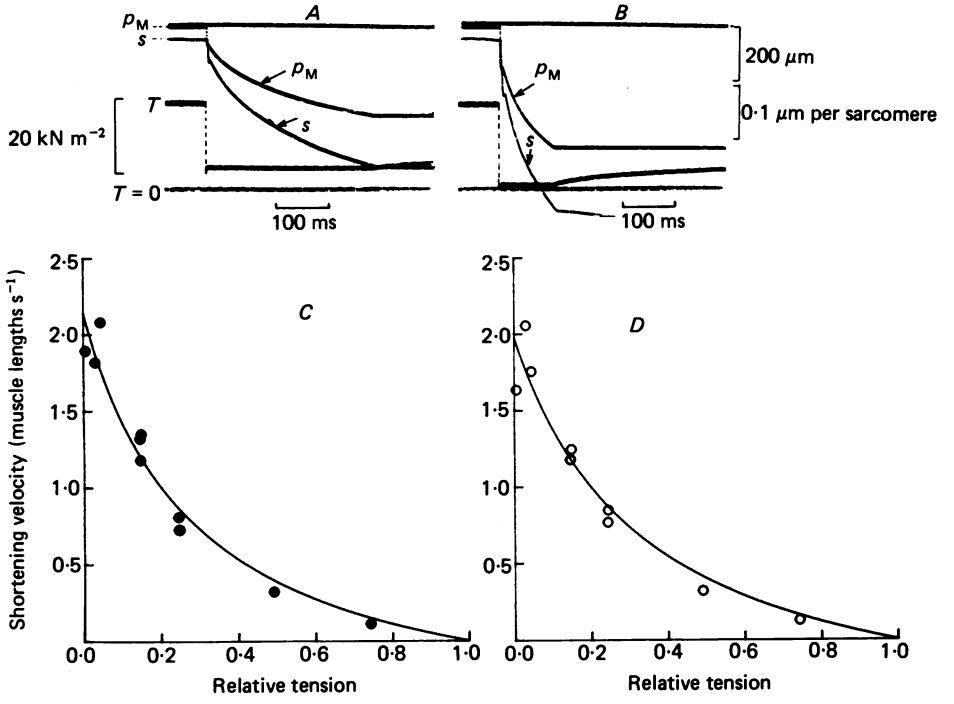


Fig. 3. Comparison of shortening velocities at 5 mM-MgATP measured from over-all length change and sarcomere length change. Fibre was partially activated (pCa $\cong$ 5·7), *A* and *B*, records showing over-all length change (motor position signal, p_M), sarcomere length signal (s) from diffraction device and tension (T) in two successive contractions with isotonic releases to $0{\cdot}24\,P_0$ (*A*) and $0{\cdot}04\,P_0$ (*B*). Calibration for p_M is in μm and for s in μm per sarcomere. *C* and *D*, force–velocity relation for this experiment with velocity measured from p_M (*C*) and from s (*D*). Fibre dimensions: $l = 3{\cdot}43$ mm, $s = 2{\cdot}42$ μm, $A = 3{\cdot}13 \times 10^{-8}$ m^2. Temperature = 1·9 °C. For *C* and *D*, V_{max} is 2·14 and 1·97 muscle lengths per second respectively, and a/P_0 is 0·28 and 0·34 respectively.

Variation of the MgATP concentration. Fig. 4 shows the tension record on a slow time base for four consecutive contraction–relaxation cycles. The first and fourth contractions were carried out in 5 mM-MgATP and the second and third contractions were at a substrate concentration of 10 μM. There was little difference between the steady tension levels in the two solutions but tension developed and relaxed more rapidly at the higher MgATP concentration. In 10 μM-MgATP no increase in the resting tension level was observed within the limit of accuracy of about $0{\cdot}01\,P_0$ imposed by variations in the surface tension on the transducer hook. Isotonic releases

were performed to 0·23 P_0 in the first two contractions (points a and b) and to about 0·01 P_0 in the last two contractions (points c and d).

Isometric tension. The effect on the isometric tension of varying the substrate concentration between 1 μM and 10 mM is shown in Fig. 5 which includes the results from two subsequent studies in this laboratory, performed in collaboration with Drs

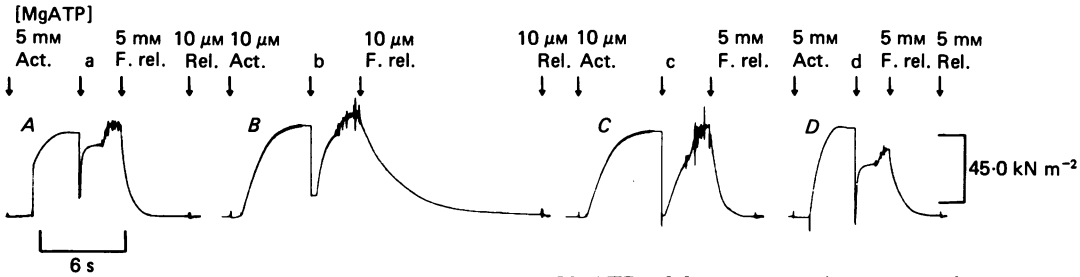


Fig. 4. Tension records at 5 mM- and 10 μM-MgATP of four consecutive contraction–relaxation cycles. The first and fourth contractions were at 5 mM-MgATP and the second and third contractions were at 10 μM-MgATP. Isotonic releases were performed during the contractions, to 0·23 P_0 (a, b) and to 0·01 P_0 (c, d). The abrupt increase of tension at the beginning of the first contraction was caused by a delay in increasing the recorder speed. Fibre dimensions: $l = 3{\cdot}02$ mm, $s = 2{\cdot}38$ μm, $A = 2{\cdot}66 \times 10^{-8}$ m². Temperature = 1·6 °C. Act., activating solution; Rel., relaxing solution; F. rel., fast relaxing solution (see Table 1).

B. B. Hamrell, J. F. Rondinone and M. Iino. The isometric tension level in the test solution was expressed relative to that in the reference solution in order to compensate for variability between fibres. Tension showed a biphasic dependence on MgATP concentration with a maximum at about 30–100 μM. At the physiological substrate concentration (5 mM), tension was about 80 % of the maximal observed tension and decreased somewhat further as the MgATP concentration was increased from 5 to 10 mM.

Force–velocity relation. Records of isotonic releases at partial calcium activation and 0·4 mM-MgATP are shown in Fig. 6*A* and *B*. The data are from the same fibre as Fig. 3 and the diffraction device was in use. The initial shortening velocity in 0·4 mM-MgATP, measured as the slope of the motor position signal (p_M) just after the tension trace became steady, was 61 % of that in 5 mM-MgATP for an isotonic release to 0·02–0·04 P_0, and 82 % for a release to 0·24–0·25 P_0. A series of tension steps for this fibre in 0·4 mM-MgATP gave the over-all length and sarcomere length data points plotted in Fig. 6*C* and *D*. Hyperbolae (continuous lines) corresponding to Hill's (1938) equation were fitted to the data points. The force–velocity curves from the p_M and sarcomere length signals again agree well. V_{max} in 0·4 mM-MgATP was 61 % of the value in 5 mM-MgATP when velocity was measured from the p_M signal and 69 % when measured from the sarcomere length signal (compare Figs. 3 and 6).

Records of isotonic releases in 5 mM- and in 10 μM-MgATP at full activation are shown in Fig. 7*A–D* (diffraction system not in use). These records are from the same contractions as those shown at a slow time base in Fig. 4. At 10 μM-MgATP, the shortening velocity was 5·5 % of that at 5 mM-MgATP for contractions *A* and *B* (releases to 0·23 P_0), and 4·8 % for contractions *C* and *D* (releases to 0·01 P_0). A series of tension steps for this fibre, in 5 mM- and 10 μM-MgATP gave the data points plotted

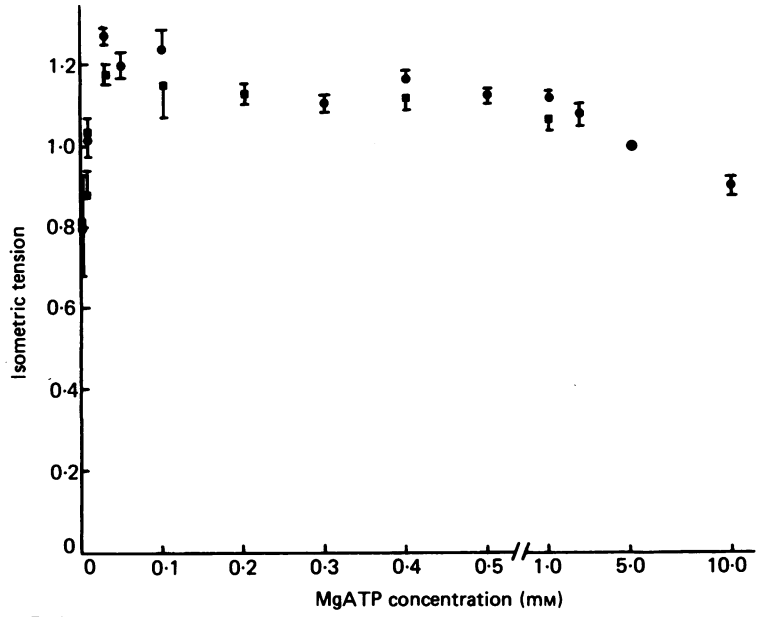


Fig. 5. Relation between isometric tension (P_0) and MgATP concentration. The average tension in two consecutive contractions in a test solution P_0 is expressed relative to the average tension in the reference solution (5 mM-MgATP) for contractions before and after those in the test solution. Note that the scale of the abscissa changes between 0·5 mM and 1·0 mM. Circles, data from the present study. Squares, data from separate studies in collaboration with Drs M. Iino, B. B. Hamrell and J. F. Rondinone. Error bars indicate ±1 S.E. of mean. Temperature 0–4 °C.

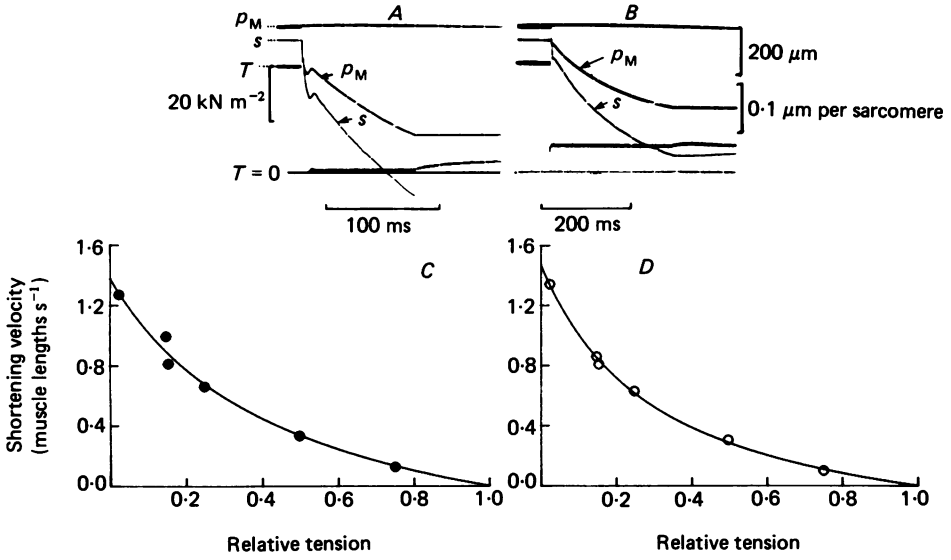


Fig. 6. Force–velocity relation at 0·4 mM-MgATP, comparing velocities measured from p_M and s records. Same fibre as in Fig. 3, partial activation, pCa ≅ 5·7. *A* and *B*, consecutive contractions in 0·4 mM-MgATP showing isotonic releases to 0·02 P_0 (*A*) and 0·25 P_0 (*B*). *C* and *D*, force–velocity relations for this experiment measured from p_M (*C*) and s (*D*). The fitted hyperbolae are characterized by $V_{max} = 1·37$ muscle lengths per second, $a/P_0 = 0·47$ for *C* and $V_{max} = 1·47$ muscle lengths per second, $a/P_0 = 0·32$ for *D*.

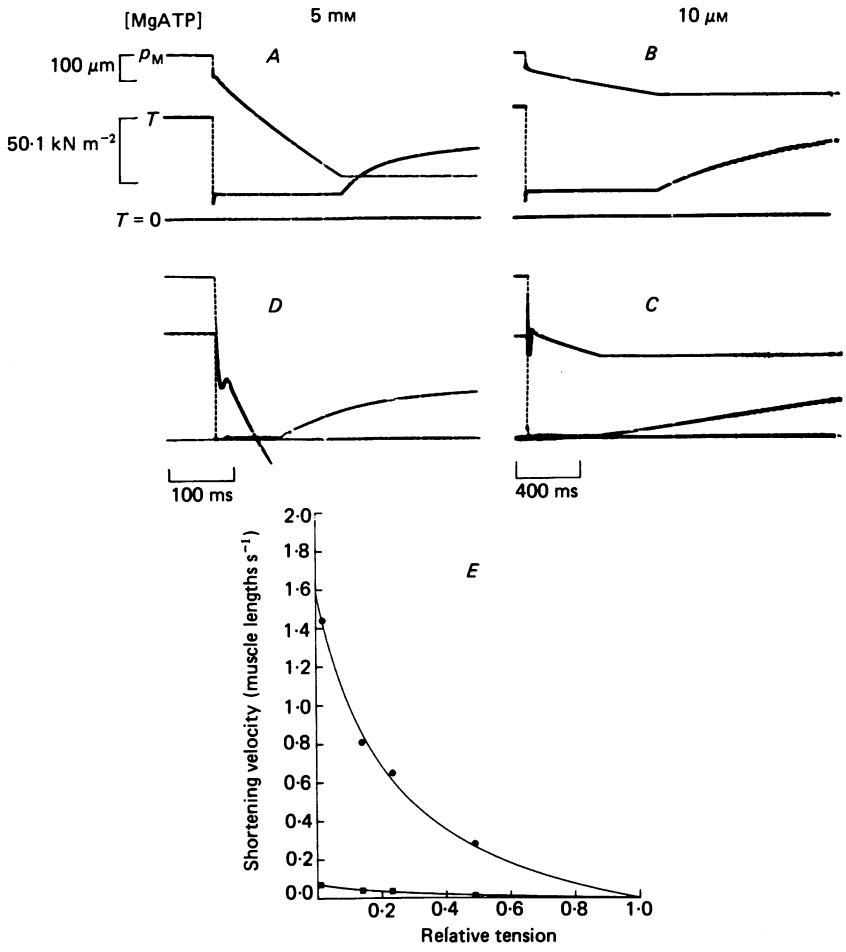


Fig. 7. Force–velocity relation in 5 mM- and 10 μM-MgATP at full activation. Same fibre as in Fig. 4. *A–D*, records of the same four isotonic releases as in Fig. 4 on a faster time base. The records were obtained in the order *A* (5 mM-MgATP), *B* (10 μM), *C* (10 μM) and *D* (5 mM). Note the difference in time scale for the two MgATP concentrations. *E*, force–velocity relations for the same fibre. Circles, 5 mM-MgATP, fitted hyperbola characterized by $V_{max} = 1{\cdot}54$ muscle lengths per second, $a/P_0 = 0{\cdot}24$. Squares, 10 μM-MgATP, fitted hyperbola characterized by $V_{max} = 0{\cdot}07$ muscle lengths per second, $a/P_0 = 0{\cdot}32$.

in Fig. 7*E*. V_{max} in 10 μM-MgATP was 4·6% of the value in 5 mM-MgATP. a/P_0 at 10 μM was 1·3 times its value in 5 mM-MgATP.

Maximum velocity of shortening. Fig. 8 shows V_{max} data for thirty-two fibres for MgATP concentrations varying from 10 μM to 10 mM at full activation. The V_{max} value from each fibre at the test MgATP concentration was plotted relative to the V_{max} value for the same fibre at 5 mM-MgATP. The relationship between V_{max} and MgATP concentration was fitted with a hyperbola. The value for K_m, the concentration at which V_{max} is half the computed asymptotic value was $0{\cdot}47 \pm 0{\cdot}14$ mM (mean $\pm$ S.E. of mean of the estimate of K_m). The V_{max} value of the fitted hyperbola extrapolated to infinitely high substrate concentration was 1·09 times the mean V_{max} value at

5 mM-MgATP. A similar curve was obtained when V_{max} was plotted directly rather than relative to the value obtained in 5 mM-MgATP, but the scatter of the data was greater. The maximal shortening velocity extrapolated to infinite substrate concentration was $2{\cdot}37 \pm 0{\cdot}11$ muscle lengths per second.

Curvature of the force–velocity relation. At substrate concentrations less than

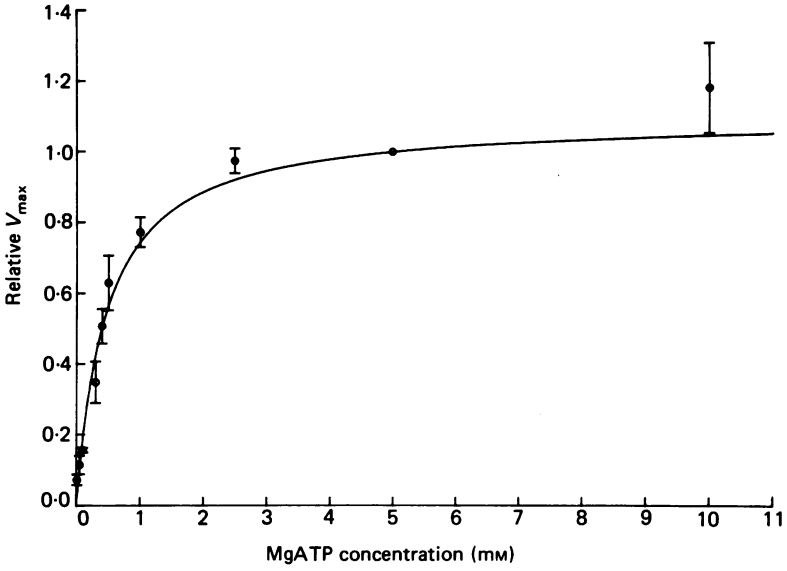


Fig. 8. Relation between the maximal shortening velocity (V_{max}) and the MgATP concentration. V_{max} is expressed relative to the value obtained for each fibre in the reference solution, 5 mM-MgATP. Error bars indicate ± 1 S.E. of mean. The data points were fitted by an hyperbola (continuous line) of the form:

$$V = V_a[\text{MgATP}]/(K_m + [\text{MgATP}]),$$

where V is the relative V_{max}, V_a is the asymptotic value for V_{max}, K_m is the substrate concentration for which the relative V_{max} is half V_a. The curve was constrained to pass through the value of 1·0 for a substrate concentration of 5 mM. The best fit was obtained for a hyperbola characterized by $K_m = 0{\cdot}47$ mM and $V_a = 1{\cdot}09$.

5 mM-MgATP, a/P_0 was found to increase slightly (Fig. 9). Although the data were scattered, the change of the curvature parameter, a/P_0, was significant (t test on the slope as described in the legend to Fig. 9). This indicates that the force–velocity relation was slightly less curved at low MgATP concentrations than at 5 mM-MgATP.

The increase of a/P_0 at low MgATP concentration was also significant when data at the test MgATP concentrations were normalized to the a/P_0 value of each fibre in the 5 mM-MgATP reference solution. However, this procedure did not decrease the scatter as was the case for the maximum shortening velocity data. Since the largest change was observed at the lowest MgATP concentration tested, we also tested the significance of the change when the 10 μM values were omitted. The slope of a/P_0 against MgATP concentration was again found to be significantly less than zero.

Control of MgATP concentration. In order to relate the observed shortening velocities directly to the MgATP concentration, the concentration in the fibre must be maintained at the same level as in the external solution, and the accumulation

of products, ADP and P_i, must be sufficiently small to have no influence on shortening velocity. The MgATP concentration was maintained by including creatine kinase and creatine phosphate in the solutions to rephosphorylate ADP produced by the myofibrillar ATPase activity. The adequacy of the rephosphorylating system for maintaining the MgATP concentration was therefore examined using shortening velocity as the test parameter.

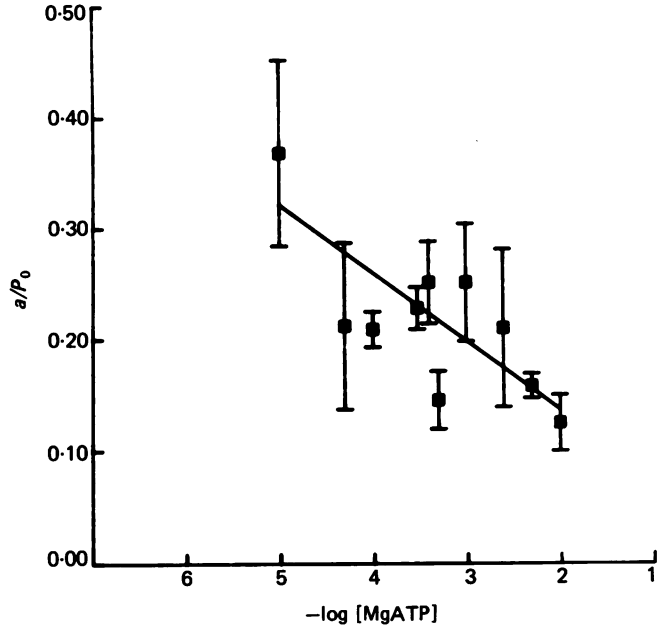


Fig. 9. Relation between a/P_0, and the negative logarithm of the MgATP concentration. Error bars indicate ± 1 S.E. of mean. The continuous line represents a linear regression to the data, with $a/P_0 = 0{\cdot}014 \pm 0{\cdot}061$ (pMgATP) for $n = 70$. A t test for the slope of the regression line gave $t(68) = 5{\cdot}045$, $P < 0{\cdot}0005$ indicating that the slope is significantly different from zero.

Fibre cross-sectional area. During a contraction the substrate concentration in the core of fibres of large diameter is more likely to fall than in thin fibres because the distance for diffusion of ATP and creatine phosphate is greater. If depletion does occur, the maximal shortening velocity of large fibres should therefore be lower than that of thin fibres. At high substrate concentration, 5 mM-MgATP, this relation between V_{max} and fibre cross-sectional area was studied for thirty-seven fibres. The data showed that there was no appreciable correlation between V_{max} and cross-sectional area (range $0{\cdot}25 \times 10^{-8}$–$2{\cdot}66 \times 10^{-8}$ m^2), although the scatter was considerable.

The relation between V_{max} and cross-sectional area was also examined in five fibres at 10 μM-MgATP. No effect of fibre cross-sectional area (range $0{\cdot}42 \times 10^{-8}$–$2{\cdot}66 \times 10^{-8}$ m^2) was observed on the absolute values of V_{max} (i.e. in muscle lengths per second), or on the V_{max} values expressed relative to those measured in 5 mM-MgATP for each fibre.

Duration of contraction. Any depletion of ATP or accumulation of products would be expected to become more severe the longer the duration of a contraction before an isotonic release. A control experiment (in collaboration with Dr M. Iino) was performed at 30 μM-MgATP in which isotonic releases to $0{\cdot}10\,P_0$ were made in successive contractions 10, 20 and 30 s after the beginning of a contraction. There was no difference in the shortening velocities in the three contractions.

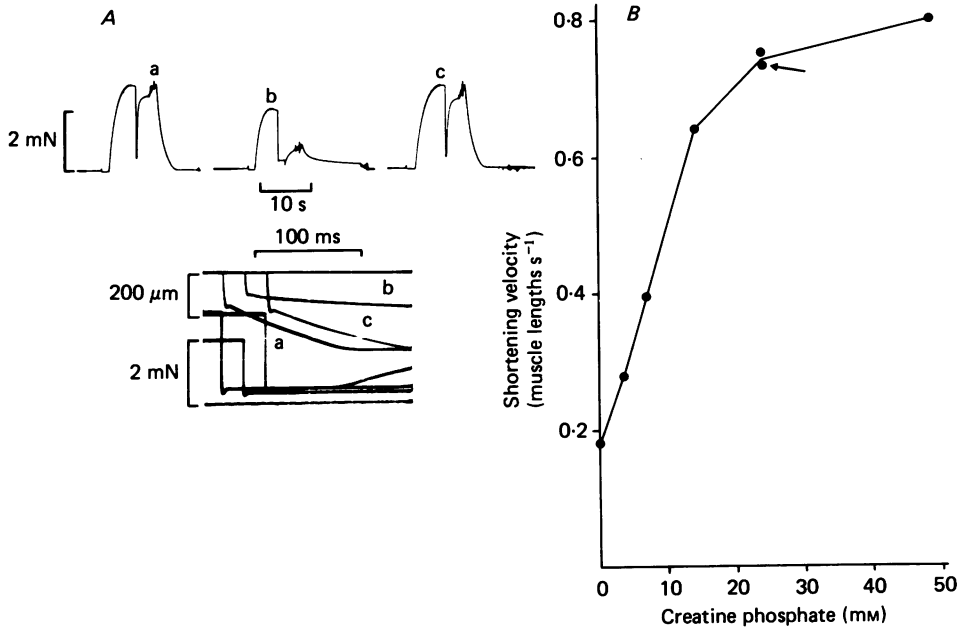


Fig. 10. Effect on shortening velocity of removal of rephosphorylating system at 0·5 mM-MgATP. *A*, oscilloscope and pen recorder records of fibre shortening in the presence (a and c) and absence (b) of creatine phosphate (19 mM) and creatine kinase (1 mg ml^{-1}). Fibre dimensions: $l = 4{\cdot}05$ mm, $s = 2{\cdot}57\,\mu$m, $A = 1{\cdot}46 \times 10^{-8}$ m^2. Temperature = 4·7 °C. *B*, relation between shortening velocity and creatine phosphate concentration at a MgATP concentration of 0·5 mM in the presence of 1 mg ml^{-1} added creatine kinase. The data points were obtained from an isotonic release to $0{\cdot}16\,P_0$. The arrow indicates a data point obtained after removal of creatine kinase. Fibre dimensions: $l = 2{\cdot}30$ mm, $s = 2{\cdot}55\,\mu$m, $A = 1{\cdot}35 \times 10^{-8}$ m^2. Temperature = 3·2 °C.

Effect of creatine phosphate and creatine kinase. At 5 mM-MgATP, total removal of the rephosphorylating system had only a small effect on the shortening velocity. However, the records of Fig. 10*A* show that at 0·5 mM-MgATP removal of creatine phosphate and creatine kinase resulted in a considerably reduced shortening velocity and that after the isotonic phase, tension redevelopment was suppressed (slow time base record, b). This indicates severe depletion of ATP in the fibre or accumulation of products of ATP hydrolysis.

At 0·5 mM-MgATP without creatine phosphate in the solutions, the shortening velocity was about one-fifth of that at saturating creatine phosphate concentration. The saturating concentration of creatine phosphate depended on the size of the fibre. In the graph of Fig. 10*B* the results from a large fibre at 0·5 mM-MgATP are shown.

Saturation of the shortening velocity required over 30 mM-creatine phosphate. The arrow indicates a data point obtained in a solution without added creatine kinase. Removal of the creatine kinase caused little decrease in the shortening velocity presumably indicating the presence of endogenous kinase.

We performed a number of further control experiments of this sort in collaboration with Dr M. Iino. A creatine phosphate concentration of 10–15 mM was found to be

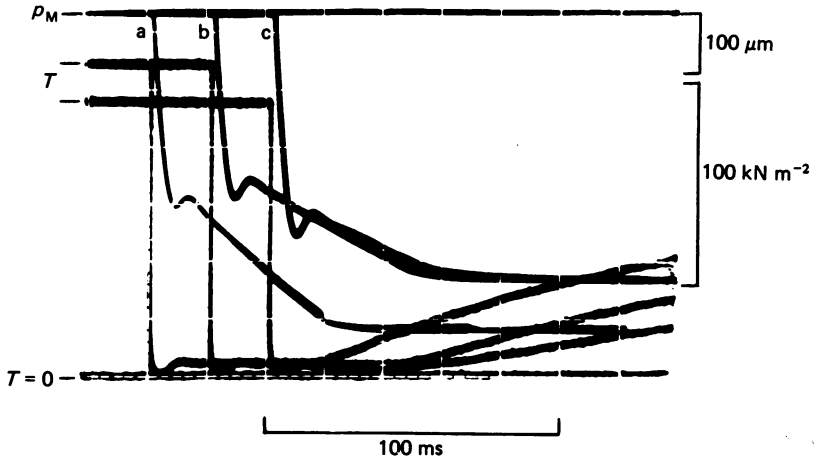


Fig. 11. Effect of excess ATP on the shortening velocity. Oscilloscope record of isotonic releases to about 0·03 P_0 in 5 mM-MgATP (a) and in two different solutions of 0·5 mM-MgATP. b, contraction in a solution where magnesium is in excess. c, contraction in solution with ATP in excess. Fibre dimensions: $l = 2·93$ mm, $s = 2·58$ μm, $A = 0·90 \times 10^{-8}$ m^2. Temperature = 4·7 °C.

adequate at 1 mM- and 30 μM-MgATP. Results for creatine kinase were more variable. In most fibres tested at MgATP concentrations between 30 μM and 5 mM, a concentration of creatine kinase of 1 mg ml^{-1} was shown to be adequate, but in two fibres at 100 μM-MgATP 6 mg ml^{-1} was required before V_{max} showed no further increase. In these fibres the velocities at 1 mg ml^{-1} were 68% and 77% of the maximum. Other fibres tested at 100 μM showed a much less marked effect of creatine kinase concentration.

Lowered free magnesium ion concentration. The solutions for the experiments described above contained an excess of magnesium over ATP. The substrate (MgATP) concentration was lowered by adding less ATP and $MgCl_2$ and the calculated free magnesium ion concentration was constant. The MgATP concentration could also be lowered by reducing the amount of $MgCl_2$ in the solutions at constant total ATP. (Table 1, solutions with excess free ATP.) In this case the free magnesium ion concentration also varied. In a control experiment (Fig. 11), a comparison was made at 0·5 mM-MgATP of the shortening velocity in the standard solution with excess added magnesium and in an excess free ATP solution at the same calculated MgATP concentration. In the excess free ATP activating solution, the free magnesium ion concentration was about 16 μM and excess ATP (not bound to magnesium) was about 5·5 mM. In the normal 0·5 mM-MgATP solution, free magnesium ion concentration was 1 mM and excess ATP was 0·1 mM. Shortening in the two solutions was slower

than in 5 mM-MgATP by about the same amount. At 0·5 mM-MgATP the rate of relaxation from a concentration was slower in an excess free ATP solution than in the normal solution. Several factors that may influence the relaxation rate are outlined in the Discussion.

For MgATP concentrations greater than 300 μM, no difference was observed in the maximal shortening velocity between the normal (excess magnesium ion) and excess ATP solutions. This indicates that the shortening velocity is limited by the MgATP concentration, not total ATP. We did not obtain data in excess ATP solutions at MgATP concentrations below 300 μM because contaminant magnesium in the TES, ATP, creatine phosphate and EGTA stocks significantly affected the MgATP concentration.

DISCUSSION

Comments on the results

Comparison with intact fibres. The reference MgATP concentration of 5 mM used in these experiments is close to the physiological concentration (Dawson, Gadian & Wilkie, 1977) so the results may be compared with the corresponding data from intact fibres of frog muscle.

The mean isometric tension, 148 kN m^{-2} at a sarcomere length of 2·56 μm is lower than that found for intact fibres from the semitendinosus muscle near 0 °C, e.g. 223 kN m^{-2} at 2·2 μm (Edman, 1979). The difference can be attributed to the swelling of the fibres, by a factor of approximately 2 in cross-sectional area, that occurs when the surface membrane is removed (Matsubara & Elliott, 1972; Godt & Maughan, 1977).

The mean value of V_{max} of 2·16 muscle lengths per second agrees well with the values, in the same units, for intact fibres of 1·95 (Edman & Hwang, 1977) and 2·47 (Edman, 1979). It also agrees well with the value of 2·47 muscle lengths per second reported for chemically skinned fibres at 4 °C (Julian, 1971). The scatter in our data is higher than that reported for intact fibres and this is probably due to differences between individual skinned fibres in the amount of damaged material at their ends (Goldman & Simmons, 1984). However, we used fibres with a wide range of diameters and it is possible that we included fibres of more than one histochemical type (Lännergren, 1975).

The value of a/P_0 of 0·16 is lower than most of the values reported in the literature for intact fibres (i.e. the force–velocity relation in skinned fibres was more curved). Edman, Mulieri & Scubon-Mulieri (1976) gave a mean value of 0·29 and in another study Edman & Hwang (1977) found a/P_0 to be 0·217 with individual values ranging from 0·160 to 0·278 (K. A. P. Edman, personal communication). An even higher value, 0·34, was given by Cecchi, Colomo & Lombardi (1978). Our value agrees well with the value of 0·18 reported by Julian & Sollins (1973) for chemically skinned fibres, so there may be a genuine difference in a/P_0 between skinned and intact fibres.

Maintenance of MgATP concentration. At least in the larger diameter fibres (2·0–2·5 $\times$ 10^{-8} m^2) used in this study, diffusion alone did not adequately maintain the concentration of MgATP within the fibres, even though the solutions were stirred vigorously to minimize boundary layers. Total removal of the rephosphorylating system even at a high MgATP concentration (5 mM) had a small but perceptible effect

on shortening velocity (not shown) and the effect at 0·5 mM was substantial (Fig. 10). At high MgATP concentration it was not necessary to add creatine kinase to the solutions because endogenous kinase was present (Walliman, Turner & Eppenberger, 1977; see below), but at low MgATP concentration at least 1 mg ml^{-1} was required for the shortening velocity to show saturation.

We performed a number of control experiments on the efficacy of the rephosphorylating system using shortening velocity as the test parameter: on the required levels of creatine kinase and creatine phosphate, on the dependence on fibre diameter and on duration of contraction, and on the effect of solutions in which free ATP was in excess. The results show that in the majority of these experiments the rephosphorylating system was adequate. In the other experiments (for example experiments with fibres of very large diameter), a deficiency in the rephosphorylating capacity may have caused a small reduction in the shortening velocity. It is unlikely that the main results were affected significantly.

At low MgATP concentration there is a possibility that the amount of free MgATP in a fibre actually increases at the start of a contraction because of a release of bound nucleotide, as the rigor state of the cross-bridges would be populated significantly. The excess MgATP would diffuse out of the fibre with a time constant of about 10 s and velocity of shortening would decrease correspondingly. The control experiment showing that shortening velocity does not vary with the time of release after the start of a contraction would seem to rule out this possibility at least at 30 μM-MgATP. However, another possibility which we have not tested is that the MgATP concentration might be affected transiently by a change of bound nucleotide brought about by isotonic shortening.

Decreased rate of tension rise and relaxation at low MgATP concentration. A consistent observation in these experiments was that tension rose more slowly in contractions at low MgATP concentrations (Fig. 4*B* and *C*) and there was an even more pronounced decrease in the rate of relaxation (Fig. 4*B*). At high MgATP concentration, the two rates are mainly determined by the diffusion of calcium and EGTA into a fibre, since the rates are slower than those in intact fibres. Some decrease in the rate of rise of tension at low MgATP concentration would be expected because a fibre has to shorten to extend the (damaged) ends and the shortening velocity is lower. The decreased relaxation rate at low MgATP concentration is not easily explained. At 10 μM-MgATP the half-time for relaxation is about 17 s, corresponding to a relaxation rate of 0·04 s^{-1}. This is very much lower than the turnover rate for ATP hydrolysis at 10 μM-MgATP which is about 0·3 s^{-1} (J. A. Sleep, personal communication). A possible explanation is that the bound nucleotide in the activated fibre was lower than in the relaxed state and 17 s represents the half-time for diffusion of additional MgATP from the bathing solution. However, other effects might prolong relaxation at low MgATP concentration, such as a change in the thin filament sensitivity to calcium ions, or a change in cross-bridge attachment due to co-operativity on the thin filament (Bremel & Weber, 1972).

Interpretation of the results

The results of this study are interpreted below in terms of the elementary steps of ATP cleavage by actomyosin. The relationship between the MgATP concentration and the isometric tension, the maximum shortening velocity and the curvature of

the force–velocity relation are discussed separately. The mechanical restraints that must exist when cross-bridges interact with actin (Hill, 1974) are discussed briefly.

Dependence of isometric tension on MgATP concentration. The biphasic dependence of isometric tension on MgATP concentration (Fig. 5) was first reported by Watanabe, Sargeant & Angleton (1964) for rabbit glycerinated fibres in the presence of calcium and it has since been observed in a number of striated muscle preparations. A similar biphasic dependence occurs for the steady-state ATPase activity of myosin in the presence of regulated actin, in the presence of calcium ions (for a review, see Murray & Weber, 1980). The effect on actomyosin ATPase activity has been explained by the co-operative binding of myosin to actin (Bremel & Weber, 1972; Bremel, Murray & Weber, 1973; Greene & Eisenberg, 1980; Trybus & Taylor, 1980). In particular, at low MgATP concentration in the absence of calcium, the ATPase activity of actomyosin is high because 'rigor complexes' (myosin attached to actin with no bound nucleotide) can form, and make neighbouring sites on actin available for binding of myosin with bound nucleotide. In the absence of calcium ions in skinned muscle fibres, the markedly biphasic relation between tension and MgATP concentration is reasonably assigned to this co-operativity (Brandt, Reuben & Grundfest, 1972; Godt, 1974).

The biphasic dependence of the actomyosin ATPase activity on MgATP concentration is most evident under conditions where the amount of myosin bound to actin is moderate. On the other hand, at saturating calcium and excess myosin the biphasic dependence is less apparent, presumably because activation is already maximal. In experiments in this laboratory, fully activated skinned fibres and myofibrils from frog and rabbit muscle have been shown to have a monotonic increase of ATPase activity with MgATP concentration that can be described by Michaelis–Menten kinetics (J. A. Sleep, personal communication). Thus the biphasic dependence of tension on MgATP concentration cannot be ascribed simply to a co-operativity in the cross-bridge turnover rate and other explanations must be sought.

A decrease of tension at high MgATP concentrations is to be expected from the dissociating effect of MgATP (Cooke & Bialek, 1979; Ferenczi, 1979). According to the simple biochemical cycle of hydrolysis of ATP by actomyosin in solution of Lymn & Taylor (1971),

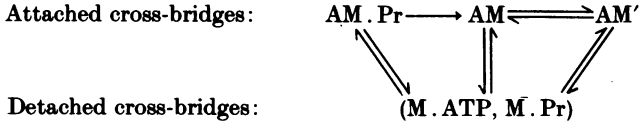
$$\begin{array}{ccc} \mathrm{AM.Pr} & \rightleftharpoons & \mathrm{AM} \\ \uparrow\downarrow & & \uparrow\downarrow \\ \mathrm{M.Pr} & \rightleftharpoons & \mathrm{M.ATP}, \end{array} \qquad (1)$$

where Pr represents the products of ATP hydrolysis, AM.Pr represents the actomyosin–products complex, progress from low to high MgATP concentration is accompanied by a shift in the predominant intermediates from AM to the other states and less myosin (M) is bound to actin (A). Thus tension can fall with increasing MgATP concentration.

The rise of tension with increase in MgATP concentration from 1 to 30 μM requires consideration of the mechanical properties of the rigor state. Several lines of evidence suggest that in rigor, the number of attached cross-bridges is high (Huxley & Brown, 1967; Thomas & Cooke, 1980), and since the tension developed in rigor is relatively low at the ionic strength and temperature of the present experiments (Y. E. Goldman & R. M. Simmons, unpublished results), each cross-bridge generates less tension in

rigor than in the presence of MgATP and calcium. In rigor a stretch applied to a frog fibre so that tension is brought to the fully activated level is followed by a decrease of tension to about $0{\cdot}3\,P_0$ with little change of stiffness (Y. E. Goldman & R. M. Simmons, in preparation). This result suggests that cross-bridges in the rigor state may slip to neighbouring actin sites, thus reducing the tension (see also Kuhn, 1978).

The effects of slippage in the rigor state on the cross-bridge cycle were considered by Ferenczi (1979) who showed that the following scheme can account for both the rise and fall of tension with increasing MgATP concentration:



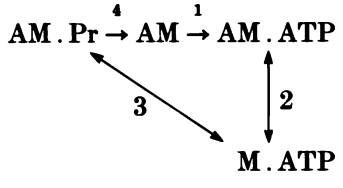
(2)

AM . Pr represents the actomyosin–products complex, a tension-generating state. The rigor state is represented by AM and AM′, and slippage is represented by the transition from AM to AM′ with a concomitant loss of tension. In the absence of MgATP, rigor tension is determined by the distribution of cross-bridges between AM and AM′. The presence of MgATP causes dissociation of the attached cross-bridges by binding either to AM or to AM′. At intermediate MgATP concentration, tension is higher than in rigor because the tension-generating states AM and AM . Pr become populated at the expense of AM′, and most cross-bridges are still attached. At high MgATP concentration, tension is lower than at intermediate MgATP concentration because a significant proportion of cross-bridges are detached.

Values for the rate constants in the scheme above can be chosen to fit the data of the rise and fall of isometric tension with increasing MgATP concentration (Fig. 5). An important constraint imposed on the values for the rate constants comes from the maximum isometric ATPase turnover rate of about 1 s^{-1} per myosin subfragment-1 (Homsher, Irving & Wallner, 1981). However, this constraint results in a second order rate constant for ATP-induced actomyosin dissociation of about 5×10^3 M^{-1} s^{-1}. This value is about two orders of magnitude lower than the value observed for actomyosin dissociation in solution (Ferenczi *et al.* 1978) and the values obtained from considerations of the dependence on MgATP concentration of shortening velocity (next section) and tension transients (unpublished results). In addition such a fit for the isometric tension results in a value for the K_m of the ATPase which is an order of magnitude greater than that measured in this laboratory (J. A. Sleep, personal communication).

A better representation of the isometric cross-bridge cycle might be obtained if additional factors were considered. For example in the above model, it is just as likely that cross-bridges would slip from the AM . Pr state as from the more tightly bound AM state. Also the model does not take into consideration the dependence of reaction rates on cross-bridge strain (Hill, 1974). In this respect we have previously discussed the possibility that cross-bridges bearing negative tension in the AM state detach preferentially, and the possibility of a force-generating AM . ATP state (Ferenczi, Simmons & Sleep, 1982).

Dependence of V_{max} on MgATP concentration. It is likely that at the maximum velocity of shortening the cross-bridge cycle most resembles the actomyosin ATPase cycle in solution. We assume that the steps leading to detachment are as suggested by studies of the interaction of myosin with actin (Finlayson, Lymn & Taylor, 1969; Lymn & Taylor, 1971) and are described by the following scheme:



(3)

It can be argued that V_{max} is primarily dependent on the rate at which cross-bridges detach (Simmons & Jewell, 1974; Ferenczi *et al.* 1982) and therefore step 3 will not markedly influence V_{max}. If we assume that cross-bridges produce force during shortening over a distance of about 10 nm (Huxley & Simmons, 1971), the maximal shortening velocity can be related to the rate of cross-bridge detachment. If cross-bridges that generate negative tension are as stiff as those generating positive tension, the average time (t) that cross-bridges spend producing negative force is roughly equal to the time spent generating positive force for the net tension to be zero. At a maximal shortening velocity of 2·37 muscle lengths per second, referred to a sarcomere length of 2·25 μm, the filament sliding velocity is: $2{\cdot}37 \times 2{\cdot}25/2 = 2{\cdot}67\ \mu\text{m s}^{-1}$ per half sarcomere and $1/t = 2{\cdot}67\ \mu\text{m s}^{-1}/10\ \text{nm} = 267\ \text{s}^{-1}$, which is an estimate of the net detachment rate.

In scheme 3, the rate of myosin dissociation from actin (AM dissociating to give A+M.ATP) is given by

$$\frac{k_2[\text{MgATP}]}{(1/K_1)+[\text{MgATP}]}$$

and shows a hyperbolic dependence on MgATP concentration. If we assign a value of 267 s^{-1} to k_2, and $1/K_1 = 0{\cdot}47$ mM (see Fig. 8) the apparent second order rate constant for AM dissociation by MgATP ($K_1 k_2$) is $267\ \text{s}^{-1}/0{\cdot}47\ \text{mM} = 5{\cdot}7 \times 10^5\ \text{M}^{-1}\ \text{s}^{-1}$, in close agreement with the observed value of $7{\cdot}4 \times 10^5\ \text{M}^{-1}\ \text{s}^{-1}$ for frog acto-subfragment-1 dissociation in solution (Ferenczi *et al.* 1978). However, a value of 375 s^{-1} for frog acto-subfragment-1 dissociation was measured at 0·5 mM-MgATP, with no sign of saturation (Ferenczi, 1979). Subsequently, even higher rates have been observed for other fast myosins. Therefore, the values of $1/K_1$ and k_2 for actomyosin dissociation are likely to be considerably higher than 0·47 mM and 267 s^{-1} respectively. There is no *a priori* reason to expect the two sets of values to be identical, because of constraints on the cross-bridges that do not exist in solution, but the agreement observed between the two second order rate constants would require that K_1 and k_2 in solution differ greatly from their values in a fibre while their product ($K_1 k_2$) is similar. This seems highly fortuitous.

Alternatively, at high MgATP concentrations, the step limiting the maximal shortening velocity could be k_4. For $k_2 \gg k_4$, the rate of detachment is given by

$$\frac{k_4[\text{MgATP}]}{(k_4/K_1 k_2)+[\text{MgATP}]}$$

and the detachment rate is half maximal for a MgATP concentration equal to $k_4/(K_1 k_2)$. With $k_4 = 267\ \text{s}^{-1}$ and $k_4/(K_1 k_2) = 0{\cdot}47$ mM the apparent second order rate constant for cross-bridge detachment in a muscle fibre, which is now $K_1 k_2$, is again equal to $5{\cdot}7 \times 10^5\ \text{M}^{-1}\ \text{s}^{-1}$. Accordingly at low MgATP concentrations the maximal shortening velocity is limited by the rate of MgATP binding, and with high MgATP it is limited by product release or an associated isomerization step.

Using the rapid photolysis of caged ATP in rabbit skinned muscle fibres, Goldman, Hibberd, McCray & Trentham (1982) have shown that cross-bridge detachment from the rigor state is rapid and little affected by the muscle tension prior to the release of MgATP. These results support our conclusion that it is not the elementary dissociation step (step 2) that limits shortening velocity.

Step 4, the putative rate limiting step for shortening velocity, is a condensation of several intermediate reactions including P_i and ADP release and isomerizations associated with these steps. Any of these reactions may depend on the cross-bridge stress or strain and account for the difference in net detachment rate between the value of about 267 s^{-1} calculated for rapid shortening and the value of about 1 s^{-1} implied for the isometric state from ATPase measurements.

Dependence of a/P_0 on MgATP concentration. a/P_0 is a measure of the curvature of the force–velocity relation, in the sense that a high value of a/P_0 gives a straighter curve. Our results suggest that a/P_0 increases slightly with decreasing MgATP concentration, in agreement with the data of Cooke & Bialek (1979) for rabbit glycerinated fibres. The rise of a/P_0 with decreasing MgATP concentration is expected from kinetic schemes in which the rate of attachment of cross-bridges is low compared to the maximum rate of detachment during shortening (Huxley, 1957). However, the observed change in curvature is less than expected if the effect of lowered MgATP concentration is simulated simply by lowering the rate of detachment (Simmons & Jewell, 1974; Ferenczi, 1979). Models in which there is a high rate of attachment are more successful in this respect (Podolsky, Nolan & Zaveler, 1969; Cooke & Bialek, 1979) but it is not clear if this is the only explanation for the result (see Ferenczi *et al.* 1982).

The cross-bridge cycle. We now consider the operation of the simplest cycle that would explain most of the results in this paper in both the isometric and isotonic states (excluding the results for isometric tension below 30 μM-MgATP, as discussed on pp. 537–538).

In scheme (3), step 4 is considered to be slow (1 s^{-1}) in the isometric state and at least 200 s^{-1} at the maximum velocity of shortening. The predominant K_m for the dependence of MgATPase activity on MgATP concentration in isometric muscle is 20 μM (J. A. Sleep, personal communication). However, if the reverse reaction rates in scheme (3) are negligible and if $k_4 = 1\ s^{-1}$ and $K_1 k_2 = 5{\cdot}7 \times 10^5\ M^{-1}\ s^{-1}$, then $K_m = k_4/(K_1 k_2) = 1{\cdot}8\ \mu M$. One possible explanation for such a discrepancy is that only a small fraction of the cross-bridges can attach to actin sites, in which case the turnover rate and hence k_4 for those cross-bridges that can attach is increased in inverse proportion. If all the cross-bridges do take part in tension generation, then the apparent discrepancy could be resolved by supposing that step 2 (or step 4) is reversible. The K_m for the ATPase activity in scheme (3) is approximately $k_4/(k_3 K_1 K_2)$. With $K_1 k_2 = 5{\cdot}7 \times 10^5\ M^{-1}\ s^{-1}$, $k_3 = 20\ s^{-1}$ (to account for the rate of rise of tension in a twitch of an intact muscle fibre) and $k_4 = 1\ s^{-1}$, then a K_m for the ATPase of 20 μM is obtained if $k_{-2} = 230\ s^{-1}$. If $1/K_1$ is high as in solution (say 5 mM), k_2 is 2850 s^{-1} and $K_2 = 12{\cdot}4$.

Further elaborations of this cycle would be necessary to account for the dependence of tension transients on MgATP concentration (Ferenczi, Goldman, Rondinone & Simmons, 1980) and for the dependence of stiffness on shortening velocity in intact fibres (Julian & Sollins, 1975; Julian & Morgan, 1981) but we confine our attention in this paper to steady-state data. In this respect the model is evidently incomplete in that it fails to account properly for the observed dependence of ATPase activity on shortening velocity in whole muscles. In isometric contractions of intact muscle, the turnover rate of ATP per myosin subfragment-1 is about 1 s^{-1} (Homsher *et al.*

1981). The turnover increases with shortening velocity reaching a peak of about 4·6 s^{-1}, but declines again at high velocities to 2·9 s^{-1} (Kushmerick & Davies, 1969; Homsher *et al.* 1981). The model described above predicts a monotonic increase in turnover rate with shortening velocity, reaching a value of 20 s^{-1} at V_{max}. The existence of a second pathway for detachment, not involving product dissociation, might explain the observed result (Podolsky & Nolan, 1973). This pathway would have much in common with the 'slippage' pathway introduced to explain the increase of tension with increasing MgATP concentration, and would not necessarily require the introduction of a wholly different concept.

Conclusions. The dependence of isometric tension on MgATP concentration does not appear to have a simple explanation and a more detailed treatment of cross-bridge mechanics will be required to account for the experimental data. On the other hand there are features of the MgATP dependence of both tension and shortening velocity that can be explained in terms of a comparatively simple biochemical cycle. Rate limitation for both shortening velocity and the turnover of MgATP in the isometric state appears to involve the release of products from attached cross-bridges or isomerizations related to product release. The equilibrium constant of the step or steps involved must depend on the tension exerted by the cross-bridges and must be up to two orders of magnitude larger in the forward direction for rapid shortening than for the isometric state. Our results suggest a close relation between cross-bridge attachment to actin sites and binding of ATP that is not apparent in models of contraction in which attachment does not occur (e.g. Iwazumi, 1979).

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